

CLAIMS

1. A method comprising:
 - a) providing:
 - i) a first recombinant vector, comprising in operable combination:
 - 1) a nucleotide sequence of interest having a 5' end and a 3' end;
 - 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest;
 - 3) adenovirus packaging sequence linked to one of said inverted terminal repeats; and
 - 4) an adeno-associated virus terminal repeat sequence operably linked to said 3' end of said nucleotide sequence of interest,
 - wherein said first vector lacks a second adeno-associated virus terminal repeat sequence, and lacks one or more adenovirus early gene region selected from E1, E2, E3, and E4 gene region; and
 - ii) a cell capable of expressing said one or more adenovirus early gene which is lacking from said first vector;
 - b) introducing said first vector into said cell to produce a transformed cell; and
 - c) culturing said transformed cell under conditions such that a second vector is produced, said second vector selected from:
 - i) a third vector, comprising in operable combination:
 - 1) adeno-associated virus terminal repeat DD sequence;

2) first and second inverted copies of a nucleotide sequence of interest flanking said adeno-associated virus terminal repeat-DD sequence;

3) left and right inverted terminal repeats of adenovirus flanking said first and second inverted copies of said nucleotide sequence of interest; and

4) an adenovirus packaging sequence linked to one of said inverted terminal repeats, and

ii) a fourth vector, comprising in operable combination:

1) a nucleotide sequence of interest having a 5' end and a 3' end;

2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest; and

3) an adenovirus packaging sequence linked to one of said inverted terminal repeats.

2. The method of Claim 1, wherein said cell is capable of expressing one or more Rep proteins, and said culturing results in expression of said one or more Rep proteins.

3. The method of Claim 1, wherein said second vector is encapsidated.

4. The method of Claim 3, further comprising d) recovering said encapsidated second vector.

5. The method of Claim 4, further comprising e) purifying said recovered encapsidated second vector.

6. The method of Claim 5, further comprising e) administering said purified encapsidated second vector to a host cell.

5 7. The method of Claim 6, wherein said administering is under conditions such that said nucleotide sequence of interest in said encapsidated second vector is expressed.

8. The method of Claim 6, wherein said host cell is a cultured cell.

10 9. The method of Claim 6, wherein said host cell is comprised in a mammal.

15 10. The method of Claim 9, wherein said mammal is selected from mouse and human.

11. The method of Claim 2, wherein expression of one or more Rep proteins is inducible.

12. A method comprising:

20 a) providing:

i) a first recombinant vector, comprising in operable combination:

25 1) a nucleotide sequence of interest having a 5' end and a 3' end;

2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest;

3) adenovirus packaging sequence linked to one of said inverted terminal repeats; and

4) an adeno-associated virus terminal repeat sequence operably linked to said 3' end of said nucleotide sequence of interest,

5 wherein said first vector lacks a second adeno-associated virus terminal repeat sequence, and lacks one or more adenovirus early gene region selected from E1, E2, and E4 gene region;

10 ii) a cell capable of expressing one or more Rep proteins; and
iii) helper adenovirus;

b) introducing said first vector and genome of said helper adenovirus into said cell to produce a transformed cell; and
c) culturing said transformed cell under conditions such that said transformed cell expresses said one or more Rep proteins, and a second vector is produced, said second vector selected from:

15 i) a third vector, comprising in operable combination:
1) adeno-associated virus terminal repeat DD sequence;
2) first and second inverted copies of a nucleotide sequence of interest flanking said adeno-associated virus terminal repeat-DD sequence;
3) left and right inverted terminal repeats of adenovirus flanking said first and second inverted copies of said nucleotide sequence of interest; and
4) an adenovirus packaging sequence linked to one of said inverted terminal repeats, and
20 ii) a fourth vector, comprising in operable combination:
1) a nucleotide sequence of interest having a 5' end and a 3' end;

- 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest; and
- 3) an adenovirus packaging sequence linked to one of said inverted terminal repeats

13. The method of Claim 12, wherein said cell lacks expression of said one or more adenovirus early gene region which is lacking from said first vector.

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14. A method comprising:

- a) providing:
 - i) a first recombinant vector, comprising in operable combination:
 - 1) a nucleotide sequence of interest having a 5' end and a 3' end;
 - 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest;
 - 3) adenovirus packaging sequence linked to one of said inverted terminal repeats; and
 - 4) an adeno-associated virus terminal repeat sequence operably linked to said 3' end of said nucleotide sequence of interest,
 - wherein said first vector lacks a second adeno-associated virus terminal repeat sequence, and lacks one or more adenovirus early gene region selected from E1, E2, and E4 gene region;
 - ii) a cell capable of expressing said one or more adenovirus early gene which is lacking from said first vector; and
 - iii) adeno-associated virus;

b) introducing said first vector and genome of said adeno-associated virus into said cell to produce a transformed cell; and

c) culturing said transformed cell under conditions such that a second vector is produced, said second vector selected from:

5 i) a third vector, comprising in operable combination:

- 1) adeno-associated virus terminal repeat DD sequence;
- 2) first and second inverted copies of a nucleotide sequence of interest flanking said adeno-associated virus terminal repeat-DD sequence;
- 3) left and right inverted terminal repeats of adenovirus flanking said first and second inverted copies of said nucleotide sequence of interest; and
- 4) an adenovirus packaging sequence linked to one of said inverted terminal repeats, and

10 ii) a fourth vector, comprising in operable combination:

- 1) a nucleotide sequence of interest having a 5' end and a 3' end;
- 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest; and
- 3) an adenovirus packaging sequence linked to one of said inverted terminal repeats.

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25 15. A method comprising:

a) providing:

- i) a first recombinant vector, comprising in operable combination:

- 1) a nucleotide sequence of interest having a 5' end and a 3' end;
- 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest;
- 3) adenovirus packaging sequence linked to one of said inverted terminal repeats; and
- 4) an adeno-associated virus terminal repeat sequence operably linked to said 3' end of said nucleotide sequence of interest,

wherein said first vector lacks a second adeno-associated virus terminal repeat sequence, and lacks adenovirus E3 early gene region; and

- ii) a cell;

- b) introducing said first vector into said cell to produce a transformed cell; and
- c) culturing said transformed cell under conditions such that a second vector is produced, said second vector selected from:
 - i) a third vector, comprising in operable combination:
 - 1) adeno-associated virus terminal repeat DD sequence;
 - 2) first and second inverted copies of a nucleotide sequence of interest flanking said adeno-associated virus terminal repeat-DD sequence;
 - 3) left and right inverted terminal repeats of adenovirus flanking said first and second inverted copies of said nucleotide sequence of interest; and
 - 4) an adenovirus packaging sequence linked to one of said inverted terminal repeats, and
 - ii) a fourth vector, comprising in operable combination:

- 1) a nucleotide sequence of interest having a 5' end and a 3' end;
- 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest; and
- 3) an adenovirus packaging sequence linked to one of said inverted terminal repeats.

16. The method of Claim 15, wherein said cell is capable of expressing one or more Rep proteins, and said culturing results in expression of said one or more Rep proteins.

17. A method comprising:

- a) providing:
 - i) a first recombinant vector, comprising in operable combination:
 - 1) a nucleotide sequence of interest having a 5' end and a 3' end;
 - 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest;
 - 3) adenovirus packaging sequence linked to one of said inverted terminal repeats; and
 - 4) an adeno-associated virus terminal repeat sequence operably linked to said 3' end of said nucleotide sequence of interest,

wherein said first vector lacks a second adeno-associated virus terminal repeat sequence, and wherein said nucleotide sequence of interest in said first vector comprises adeno-associated virus rep gene region; and

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- ii) a cell;
- b) introducing said first vector into said cell to produce a transformed cell; and
- c) culturing said transformed cell under conditions such that said transformed cell expresses one or more Rep proteins, and a second vector is produced, said second vector selected from:
 - i) a third vector, comprising in operable combination:
 - 1) adeno-associated virus terminal repeat DD sequence;
 - 2) first and second inverted copies of a nucleotide sequence of interest flanking said adeno-associated virus terminal repeat-DD sequence;
 - 3) left and right inverted terminal repeats of adenovirus flanking said first and second inverted copies of said nucleotide sequence of interest; and
 - 4) an adenovirus packaging sequence linked to one of said inverted terminal repeats, and
 - ii) a fourth vector, comprising in operable combination:
 - 1) a nucleotide sequence of interest having a 5' end and a 3' end;
 - 2) left and right inverted terminal repeats of adenovirus flanking said nucleotide sequence of interest; and
 - 3) an adenovirus packaging sequence linked to one of said inverted terminal repeats.

18. The method of Claim 17, wherein said first vector lacks one or more adenovirus early gene region selected from E1, E2, and E4 gene region, and said cell

is capable of expressing said adenovirus early gene region which is lacking from said first vector.

19. The method of Claim 17, wherein said first vector lacks adenovirus E3
5 gene region.